Neurorehabilitation and Neural Repair
http://nnr.sagepub.com/

Nerve Growth Factor Eye Drop Administration Improves Visual Function in a Patient With Optic Glioma

Antonio Chiaretti, Benedetto Falsini, Serenella Servidei, Dario Marangoni, Filomena Pierri and Riccardo Riccardi

Neurorehabil Neural Repair 2011 25: 386 originally published online 22 February 2011
DOI: 10.1177/1545968310395601

The online version of this article can be found at:
http://nnr.sagepub.com/content/25/4/386

Published by:
SAGE
http://www.sagepublications.com

On behalf of:

American Society of Neurorehabilitation

Neurorehabilitation and Neural Repair
25(4) 386–390

DOI: 10.1177/1545968310395601
http://nnr.sagepub.com
SAGE

Nerve Growth Factor Eye Drop Administration Improves Visual Function in a Patient With Optic Glioma

Antonio Chiaretti, MD [1], Benedetto Falsini, MD[1], Serenella Servidei, MD[1], Dario Marangoni , MD[1], Filomena Pierri, MD[1], and Riccardo Riccardi, MD[1]

Abstract

Background. Spontaneous visual improvement in people with an optic glioma (OG) of the anterior or retrochiasmatic optic pathways is rare. *Objective.* To evaluate the effects on visual function of nerve growth factor (NGF) eye drop administration in a patient with severe visual impairment due to a low-grade OG. *Methods.* A 45-year-old woman with OG and long-standing optic nerve atrophy was assessed before and after 2 NGF treatment courses. The drug used was 2.5S murine NGF. One milligram of NGF, diluted in saline solution, was administered onto the conjunctiva of both eyes for 10 consecutive days 3 times a day for each treatment. The follow-up was performed by clinical, neuroradiologic, and electrophysiological tests (electroretinogram and visual evoked potentials [VEPs]) at the end of each treatment and 30 and 60 days later. *Results.* A repeated subjective and objective improvement of visual function (>3 lines visual acuity; >40° visual field; >50% VEP amplitude increase, Wilcoxon test $P < .01$) was recorded after NGF treatment. These measures tended to deteriorate toward baseline values 60 days from the end of each NGF treatment. No ocular or systemic side effects were observed throughout treatment. *Conclusions.* Conjunctival NGF may be a beneficial adjunct therapy for visual loss in patients with OG, possibly exerting its effects on residual viable optic pathways.

Keywords

nerve growth factor, neuroprotection, optic gliomas, vision loss

Introduction

Several neoplastic diseases, such as craniopharyngioma and optic glioma (OG), can compromise visual function involving both anterior and retrochiasmatic optic pathways. No specific therapy is currently available for OG-induced visual loss. Nerve growth factor (NGF) is the first discovered neurotrophin involved in the development and survival of sympathetic, sensory, and forebrain cholinergic neurons.[1] In experimental animal models, NGF promotes nerve terminal outgrowth and neuron recovery after inflammatory, ischemic, and toxic injuries.[2] Given its actions favoring neuronal survival, NGF has been proposed for the treatment of some traumatic, ischemic, and neurodegenerative brain diseases.[3,4] Intraventricular NGF administration may ameliorate symptoms in adults with Parkinson's and Alzheimer's disease and improves cerebral blood flow in infants with hypoxic–ischemic brain injury.[5-8] Exogenous NGF showed neuroprotective effects also on the visual system[9] because of the presence of NGF receptors on the conjunctiva, cornea, and retinal ganglion cells (RGCs).[10] The effects of NGF and other neurotrophins, such as brain-derived neurotrophic factor (BDNF), are mediated via uptake by RGCs, anterograde transport along the optic nerve, and release to the postsynaptic geniculate neurons.[11] In experimental animal models, intraocular NGF administration keeps RGCs from degeneration after optic nerve transection and ocular ischemia and leads to the rescue of axotomized forebrain cholinergic neurons,[12,13] whereas conjunctivally applied NGF proved to be effective in patients with corneal ulcers and severe glaucoma.[14,15] Indeed, it has been reported that NGF administered to the

[1]Catholic University of Rome, Rome, Italy

Corresponding Author:
Antonio Chiaretti, MD, Department of Pediatric Neuroscience, Catholic University of Rome, Largo A Gemelli 8, Rome 00168, Italy
Email: achiaretti@yahoo.it

conjunctiva rapidly reaches the retina and the optic nerve where this neurotrophin exerts its biological activities through the uptake by RGCs.[16] Based on these evidences, we report the first known case of NGF effect on visual loss reversal in a patient with OG.

Case Report

A 45-year-old woman, affected since childhood by genetically confirmed neurofibromatosis type I, had a diagnosis of anterior prechiasmatic OG at age 33 years with bilateral and steadily progressive deterioration of both central and peripheral vision. At admission neuro-ophthalmologic examination showed bilateral proptosis of 3 mm, normal extrinsic and intrinsic ocular motility. Best corrected visual acuity was as follows: right 0.2 (−6 sph), left 0.1 (−8 sph); near distance: 5th size (Jaeger reading chart) bilaterally. Ishihara plate testing showed bilateral red-green axis dyschromatopsia. Goldmann visual field testing showed bilateral concentric, severe constriction with both V/4e and II/4e isopters (main diameter = 15° and 10°, respectively). Visual evoked potentials (VEPs) pattern reversal and flicker (8 Hz) stimulation showed severe bilateral amplitude attenuation with an increased time-to-peak. Electroretinograms (ERGs) showed attenuated amplitudes bilaterally of the inner retina–related flicker 1st and 2nd harmonic components.

Based on the role of NGF in important biochemical and molecular mechanisms of the optic pathways, we decided to treat this patient by NGF eye drop administration when marked and progressive visual loss was no longer responsive to conventional treatment with steroids and mannitol. The second NGF course was carried out after 60 days in the same fashion, according to an institutional review board/ethical committee–approved protocol.

The drug used was 2.5S NGF, purified and lyophilized from male mouse submandibular glands and prepared according to the method of Bocchini and Angeletti.[17] The submandibular glands of adult male mice were explanted under sterile conditions and the tissues were homogenized, centrifuged, and dialyzed. This aqueous gland extract was then passed through subsequent cellulose columns, thereby separating NGF by adsorption. The first step was gel filtration at pH 7.5, wherein most of the active NGF was eluted in the 80 000 to 90 000 molecular weight range. The samples obtained were analyzed by spectrophotometry at a wavelength of 280 nm to identify NGF-containing fractions. Specificity of fractions was determined by Western blot analysis. NGF purity (>95%) was estimated by high-performance liquid chromatography column equipped with a guard column calibrated with 40 mg of purified and bioactive murine 2.5S NGF standard. The NGF obtained was then dialyzed and lyophilized under sterile conditions and stored at −20° until used. Biological activity of purified NGF was evaluated by in vitro stimulation of neurite outgrowth in rat pheochromocytoma PC12 cells over a period of 7 to 14 days. NGF was dissolved in 0.9% sterile saline solution in concentrations of 200 μg/mL. The concentration of NGF in this solution was stable for the entire treatment time. One milligram of NGF, diluted in 5 mL of saline solution (0.2 mg/mL), was administered onto the conjunctiva of both eyes for 10 consecutive days 3 times a day for each treatment. This dose is considered sufficient to reach and stimulate NGF receptors in most cerebral cholinergic areas of the brain and optic pathways, as previously reported in the literature.[5]

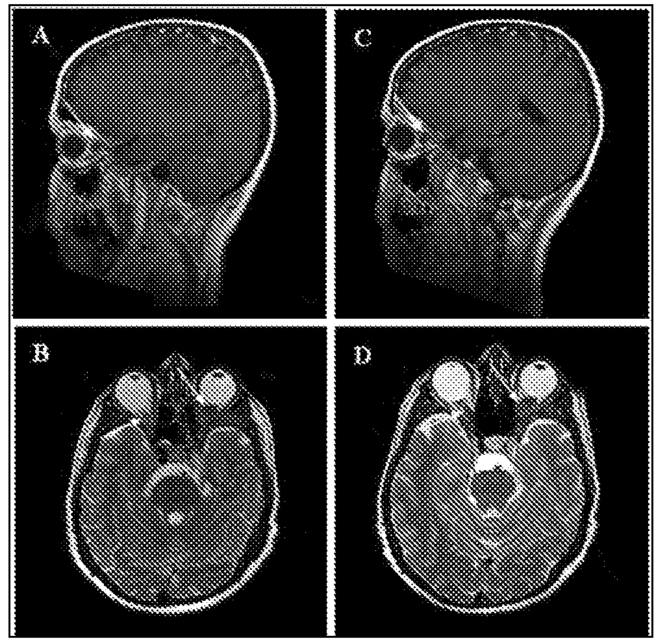

Figure 1. Sagittal T1-weighted magnetic resonance imaging scan (MRI) of the brain and orbits showing the glioma affecting the left optic nerve (white arrow) at baseline (A) and 4 months after nerve growth factor (NGF) administration (C). Axial T2-weighted MRI showing both right and left optic nerves involved by bilateral optic gliomas (white arrow) at baseline (B) and 4 months after NGF administration (D). No significant change in tumor volume after NGF treatment was found

No ocular or systemic side effects occurred. Brain magnetic resonance imaging (MRI) evaluation, performed at baseline and after 120 days, did not show any significant change in tumor volume after NGF treatment (Figure 1 A-D). The follow-up was performed by clinical and electrophysiological tests at the end of each treatment and after further 30 and 60 days. A subjective and objective improvement of visual acuity and visual field (>3 lines visual acuity; >40° visual field; Wilcoxon test, $P < .01$; magnitude of change >99 percentile of the test–retest variability for both visual acuity and visual field[18,19]; Figure 2A and B) allowing her to walk confidently, watch TV, and recover the customary activities was recorded after 48 hours from the

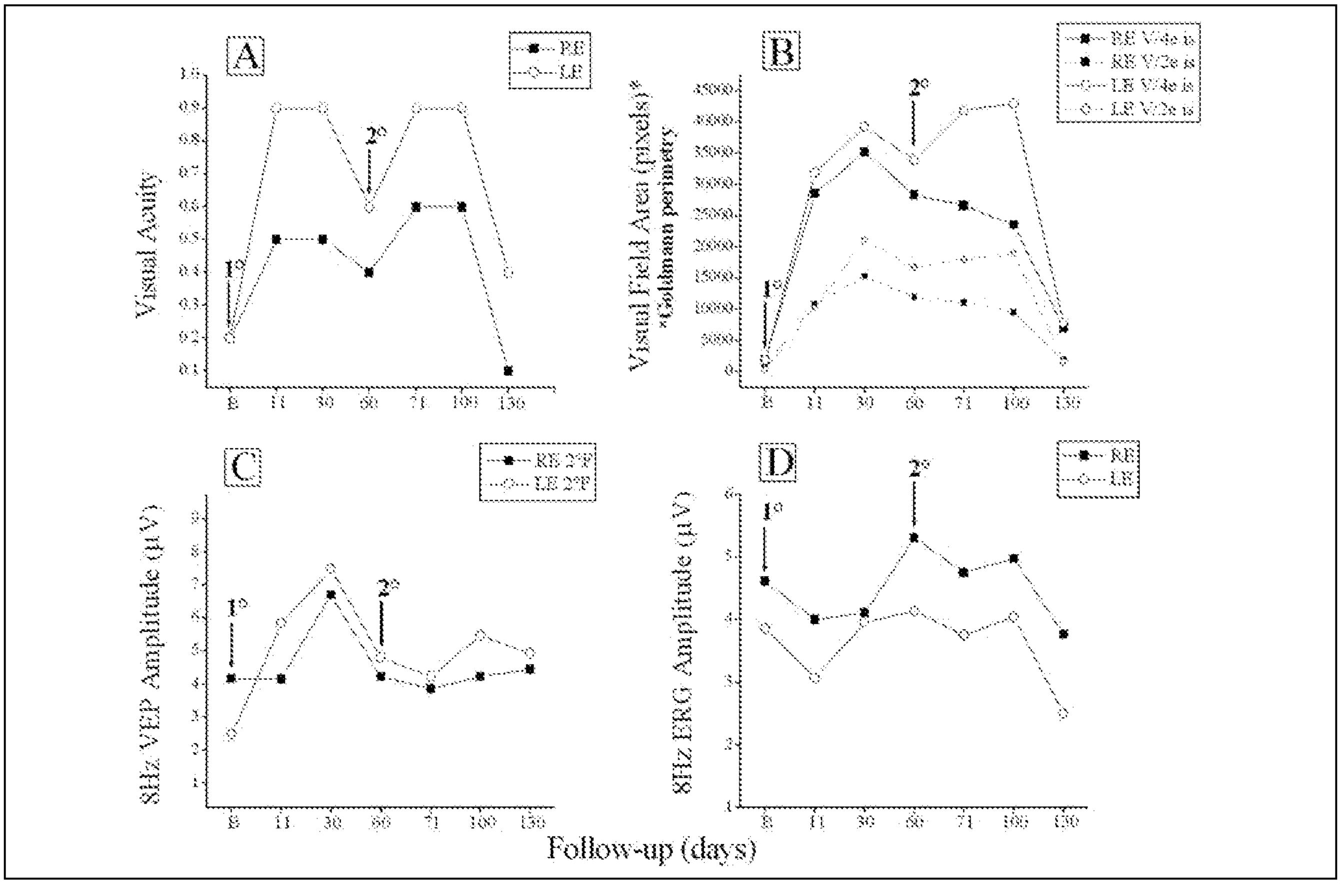

Figure 2. Right after the end of each treatment, visual acuity (A) recovered in both eyes by 3 to 7 lines (significant [$P < .05$] increase or decrease is ≥2 lines). (B) Lateral visual fields reported in relative units following planimetric analysis, recovered by several log units of area indicating a main increase in lateral perception of 40° in RE and 35° in LE. (C) VEPs showed an increase in amplitude in parallel with visual acuity improvement. (D) ERGs showed amplitude enhancement 60 days after the first NGF course and then tended to decline

Abbreviations: B, baseline; ERG, electroretinogram; LE, left eye; NGF, nerve growth factor; 1°, first NGF administration; 2°, second NGF administration; RE, right eye; VEP, visual evoked potential.

beginning of each treatment. Also, VEPs and ERGs showed an improvement after NGF administration (>50% increase in VEP amplitude; changes >99 percentile of the test–retest variability[20]; Figure 2C and D). Visual acuity, visual field, and VEPs tended, following the improvement, to deteriorate toward baseline values after 60 days from the end of each NGF treatment, suggesting the need to continue NGF administration every 2 months.

Discussion

This report shows that NGF eye drop administration may ameliorate visual acuity, visual field, VEPs, and ERGs amplitudes in this patient with OG, as indicators of visual loss reversal after NGF treatment. The improvement of VEPs and ERGs suggests molecular events within surviving RGCs leading to a recovery of their physiological responses. It is known that RGCs express NGF receptor (TrkA) and that NGF, binding to TrkA, upregulates Bcl-2 protein, which protects cells from apoptosis by preventing caspase activation.[21] Furthermore, intravitreal NGF delivery to the retina and optic nerve is crucial to the survival of RGCs and for functional recovery of the retina following ocular ischemia and hypertension in experimental animal models.[13,22] Last, conjunctivally applied NGF has been shown to reach sharply the retina and optic nerve where this neurotrophin exerts its biological activities.[16] In our patient, the electrofunctional changes after NGF administration were immediately followed by clinical improvement. These effects confirm the key role of neurotrophins in modulating RGC function and visual cortical neuronal activity, as reflected by receptive field size and response latency.[23,24] Amelioration of both clinical and electrophysiological parameters lasted for about 60 days after NGF treatment, indicating that NGF effect on optic pathways had a prolonged duration. This prolonged and stable NGF effect may

be related not only to a protective activity against neural apoptosis but also to the formation of new neural pathways, since it is known that NGF promotes neuronal repair and axonal regeneration.[25,26] NGF acts on different levels to promote neuronal recovery following ischemic, inflammatory, and traumatic injuries: through a neosynaptogenetic mechanism, by directly affecting precursor cells, and/or by induction of other growth factors, such as BDNF, whose neuroprotective effects on visually evoked RGC responses after optic nerve section have been reported.[11,27]

Caution is necessary. NGF promotes proliferative activity of glioblastoma cells through direct interaction with activation of RAS and MAPK Erk1/2 pathway.[28] The level of NGF increases in the malignant glioma tissue,[29] although the opposite has been found for low-grade astrocytomas,[30] whereas growth control of glioma by NGF has been shown in vivo.[31] Currently, drugs that block RAS and mTOR are under investigation for treatment of NF1-associated malignancies (see www.clinicaltrials.gov). Potential complications of long-term therapy using topical NGF treatment on NF1-associated OG include an increase in tumor size. A recent 6-month, phase I trial of topical NGF in children with OG from our group did not show any growth by MRI.

Prospective and controlled clinical trials should be conducted to explore the efficacy of conjunctivally applied NGF on optic nerve function. In parallel, animal studies could identify NGF-induced mechanisms in genetically engineered mice with knockout of the NF-1 gene that develop OG.[32] These animal models could evaluate the changes and correlations between NGF and other neurotrophic factors, such as BDNF, basic fibroblast growth factor, ciliary neurotrophic factor, and synaptic vesicle modifications.

Authors' Note

Antonio Chiaretti and Benedetto Falsini contributed equally to this study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

Funding

The author(s) received no financial support for the research and/or authorship of this article.

References

1. Levi-Montalcini R. The nerve growth factor 35 years later. *Science*. 1987;874:1154-1162.
2. DeKosky ST, Taffe KM, Abrahamson EE, Dixon CE, Kochanek PM, Ikonomovic MD. Time course analysis of hippocampal nerve growth factor and antioxidant enzyme activity following lateral controlled cortical impact brain injury in the rat. *J Neurotrauma*. 2004;21:491-500.
3. Chiaretti A, Barone G, Riccardi R, et al. NGF, DCX, and NSE upregulation correlates with severity and outcome of head trauma in children. *Neurology*. 2009;72:609-616.
4. Mufson EJ, Kroin JS, Sendera TJ, Sobreviela T. Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. *Prog Neurobiol*. 1999;57:451-484.
5. Eriksdotter Jönhagen M, Nordberg A, Amberla K, et al. Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. *Dement Geriatr Cogn Disord*. 1998;9:246-257.
6. Olson L, Backlund EO, Ebendal T, et al. Intraputaminal infusion of nerve growth factor to support adrenal medullary autografts in Parkinson's disease. One-year follow-up of first clinical trial. *Arch Neurol*. 1991;48:373-381.
7. Chiaretti A, Antonelli A, Genovese O, et al. Intraventricular nerve growth factor infusion improves cerebral blood flow and stimulates doublecortin expression in two infants with hypoxic-ischemic brain injury. *Neurol Res*. 2008;30:223-228.
8. Chiaretti A, Genovese O, Riccardi R, et al. Intraventricular nerve growth factor infusion: a possible treatment for neurological deficits following hypoxic-ischemic brain injury in infants. *Neurol Res*. 2005;27:741-746.
9. Micera A, Lambiase A, Aloe L, Bonini S, Levi-Schaffer F, Bonini S. Nerve growth factor involvement in the visual system: implications in allergic and neurodegenerative diseases. *Cytokine Growth Factor Rev*. 2004;15:411-417.
10. Carmignoto G, Comelli MC, Candeo P, et al. Expression of NGF receptor mRNA in the developing and adult rat retina. *Exp Neurobiol*. 1991;111:302-311.
11. Caleo M, Menna E, Chierzi S, Cenni MC, Maffei L. Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system. *Curr Biol*. 2000;10:1155-1161.
12. Carmignoto G, Maffei L, Candeo P, Canella R, Comelli C. Effect of NGF on the survival of rat retinal ganglion cells following optic nerve section. *J Neurosci*. 1989;9:1263-1272.
13. Siliprandi R, Canella R, Carmignoto G. Nerve growth factor promotes functional recovery of retinal ganglion cells after ischemia. *Invest Ophthalmol Vis Sci*. 1993;34:3232-3245.
14. Lambiase A, Rama P, Bonini S, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for corneal neurotrophic ulcers. *N Engl J Med*. 1998;338:1174-1180.
15. Lambiase A, Aloe L, Centofanti M, et al. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: implications for glaucoma. *Proc Natl Acad Sci U S A*. 2009;106:13469-13474.
16. Lambiase A, Tirassa P, Micera A, Aloe L, Bonini S. Pharmacokinetics of conjunctivally applied nerve growth factor in the retina and optic nerve of adult rats. *Invest Ophthalmol Vis Sci*. 2005;46:3800-3806.
17. Bocchini V, Angeletti PU. The nerve growth factor: purification as a 30,000 molecular weight protein. *Proc Natl Acad Sci U S A*. 1969;64:787-794.

18. Stifter E, König F, Lang T, et al. Reliability of a standardized reading chart system: variance component analysis, test-retest and inter-chart reliability. *Graefes Arch Clin Exp Ophthalmol*. 2004;242:31-39.
19. Ross DF, Fishman GA, Gilbert LD, Anderson RJ. Variability of visual field measurements in normal subjects and patients with retinitis pigmentosa. *Arch Ophthalmol*. 1984;102:1004-1010.
20. Falsini B, Ziccardi L, Lazzareschi I, et al. Longitudinal assessment of childhood optic gliomas: relationship between flicker visual evoked potentials and magnetic resonance imaging findings. *J Neurooncol*. 2008;88:87-96.
21. Coassin M, Lambiase A, Sposato V, Micera A, Bonini S, Aloe L. Retinal p75 and bax over expression is associated with retinal ganglion cells apoptosis in a rat model of glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:1743-1749.
22. Lambiase A, Centofanti M, Micera A, et al. Nerve growth factor (NGF) reduces and NGF antibody exacerbates retinal damage induced in rabbit by experimental ocular hypertension. *Graefes Arch Clin Exp Ophthalmol*. 1997;235:780-785.
23. Pizzorusso T, Porciatti V, Tseng JL, Aebischer P, Maffei L. Transplant of polymered capsulated cells genetically engineered to release nerve growth factor allows a normal functional development of the visual cortex in dark-reared rats. *Neuroscience*. 1997;80:307-311.
24. Rohrer B, Korenbrot JI, LaVail MM, Reichardt LF, Xu B. Role of neurotrophin receptor TrkB in the maturation of rod photoreceptors and establishment of synaptic transmission to the inner retina. *J Neurosci*. 1999;19:8919-8930.
25. Lu B. Acute and long-term synaptic modulation by neurotrophins. *Prog Brain Res*. 2004;146:137-150.
26. Sofroniew MV, Howe CL, Moblley WC. Nerve growth factor signaling, neuroprotection, and neural repair. *Annu Rev Neurosci*. 2001;24:1217-1281.
27. Chen H, Weber AJ. BDNF enhances retinal ganglion cell survival in cats with optic nerve damage. *Invest Ophthalmol Vis Sci*. 2001;42:966-974.
28. Brown MC, Staniszewska I, Lazarovici P, Tuszynski GP, Del Valle L, Marcinkiewicz C. Regulatory effect of nerve growth factor in alpha9beta1 integrin-dependent progression of glioblastoma. *Neuro Oncol*. 2008;10:968-980.
29. Li JT, Yan Q, Yu HL. Expression of VEGF and NGF in gliomas of human. *Sichuan Xue Xue Bao Yi Xue Ban*. 2009;40:408-411.
30. Chiaretti A, Aloe L, Antonelli A, et al. Neurotrophic factor expression in childhood low-grade astrocytomas and ependymomas. *Childs Nerv Syst*. 2004;20:412-419.
31. Kimura S, Yoshino A, Katayama Y, Watanabe T, Fukushima T. Growth control of C6 glioma in vivo by nerve growth factor. *J Neurooncol*. 2002;59:199-205.
32. Hegedus B, Hughes FW, Garbow JR, et al. Optic nerve dysfunction in a mouse model of neurofibromatosis-1 optic glioma. *J Neuropathol Exp Neurol*. 2009;68:542-551.